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The Influence of an Acidifier Feed Additive on Biochemical Parameters and Immune Response of Broilers

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Authors' contributions

This work was carried out in collaboration between all authors. Author MM designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors MH and MY managed the analyses of the study. Author AA managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: Dietary acidifiers appear to be a possible alternative to feed antibiotics in order to improve performance of broilers. It is generally known that dietary acidifiers lower gastric pH, resulting in increased activity of proteolytic enzymes, improved protein digestibility and inhibiting the proliferation of pathogenic bacteria in GI tract. The present paper assesses the different dosage of an acidifier on commercial broilers.
Study Design: Two hundred and Forty day-old chicks were randomly distributed in a completely randomized experimental design with four treatments and three replications of twenty chicks each. Diets prepared without additive as Control (CON) (group1); 0.025% Acidifier Agent (AA1) (group2); 0.05% Acidifier Agent (AA2) (group3) and 0.1% Acidifier Agent (AA3) (group4).
Place and Duration of Study: Department of Animal Science, Malayer University, Malayer, Iran, between May 2013 and September 2013.
Methodology: At the end of the trials, six birds from each replicate were sacrificed by cutting the jugular vein and blood samples were individually collected in 10-mL heparinized tubes and stored on ice for hematological analysis. Serum was separated after 8 to 10 hours and was stored at –20ºC for subsequent analysis. The individual serum samples were analyzed for antibody titers against Newcastle disease (ND), Infectious Bursal

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Disease (IBD) and Avian Influenza (AI) by ELISA technique and using an automatic analyzer. Treatment-wise means of titers were computed. The collected blood samples were analyzed for total proteins, serum albumin, uric acid and the activities of gamma glutamyltransferase (GGT) and alanine amino transferase (ALT).

Results: Results showed that there was no significant difference among the dietary treatments for all antibody titers again Newcastle Disease (ND), Infectious Bursal Disease (IBD) and Avian Influenza (AI). The results of total protein, serum albumin and serum globulin showed no significant difference among the dietary treatments for these parameters. Activities of serum gamma glutamyltransferase (GGT), alanine amino transferase (ALT) and Alkaline phosphatase (ALP) also remained non-significant.

Conclusion: It can be concluded that dietary acidifier agent did not have a clear positive effect on immune response and serum biochemical levels; however, there was a slight positive effect on 0.1 % level of inclusion in the diet.

Keywords: Acidifier; MOS; lactic acid, ALT; GGT; immunity; broilers.

1. INTRODUCTION

Due of the concerns of antibiotic resistance and the implications for human health, there is a clear need for safe alternatives to antibiotic growth promoters in the poultry industry. To date, there have been numerous reports on the ability of enzymes [1], organic acids [2] and oligosaccharides [3] to act as growth promoters in broilers. The acidifiers - organic acids or their salts - are naturally occurring substances, many of which play an important role in the metabolism [4]. Such acids have been used for the sanitation of animal feed for decades. When these substances are included in the feed, they can modify the pH of both the feed and the digestive tract of farm animal. Also, the organic acids in their un-dissociated form are able to pass through the bacterial cell membrane inside the cell, where they dissociate in H+ ions which lower the pH of the cell and RCOO- ions that can disrupt the normal cell function and protein synthesis. As a result, the affected microorganisms are unable to replicate efficiently and the microflora of the digestive tract is modified [5,6]. The potential of single organic acids in feed preservation lies in their ability to protect feed from microbial and fungal destruction, and its effect on stomach pH and gut flora, and has been known for decades and proven in many laboratory and field trials [7,8]. Acidifiers act as performance promoters by lowering the pH of gut (mainly upper intestinal tract), reducing potential proliferation of unfavourable microorganisms. Acidification of gut stimulates enzyme activity and optimises digestion and the absorption of nutrients and minerals. Un-dissociated forms of organic acids penetrate the lipid membrane of bacterial cells and dissociate into anions and protons. After entering the neutral pH of the cell's cytoplasm, organic acids inhibit bacterial growth by interrupting oxidative phosphorylation and inhibiting adenosine triphosphate in organic phosphate interactions. Improved broiler performance by supplementation with single acids was noticed for formic acid [9] and fumaric acid [10], and Izat et al. [11] found significantly reduced levels of Salmonella spp. in carcass and caecal samples after including calcium formate to broiler diets. In another trial from Izat et al. [12] buffered propionic acid was used to counteract pathogenic microflora in the intestine and carcass of broiler chickens, and resulted in a significant reduction in E. coli and Salmonella spp. The use of pure formic acid in breeder feed reduced the contamination of tray liners and hatchery waste with S. enteritidis drastically [13]. Kirchgessner et al. [14] found significantly better feed utilization in laying hens after adding fumaric acid, but only when the feed was low in protein and methionine and cysteine. Performance enhancement was influenced by both quantity and

quality of the protein, although these trials were performed either with single organic acids or with the corresponding salt of a single acid. Hinton and Linton, [15] examined controlling salmonella infections in broiler chickens by using a mixture of formic and propionic acid. They demonstrated that under experimental conditions 6 kg/t of that organic acid blend was effective in preventing intestinal colonization with *Salmonella* spp. from naturally or artificially contaminated feed. In another study Lückstädt et al. [6] used an acid blend for one-day-old chicken. The acidifier treatment (a combination of formic and propionic acid and their salts, based on an inorganic sequential release medium) was added at a dosage rate of 3 kg/t feed. Mannan oligosaccharide (MOS) is a mannan-based carbohydrate extracted from the outer cell wall of the yeast *Saccharomyces cerevisiae* [16]. Several studies have demonstrated the benefits of adding MOS to broiler diets, such as improved gut morphology in features such as villus length and villus area [17], growth performance characteristics such as BW, feed conversion rate, and apparent ME [18]. Adding MOS to the poultry diet also exhibited beneficial changes in mucin secretion and in number of goblet cells per villus [19], in digestibility and enzyme activity [20], and in gut immune responses [21]. Furthermore, MOS has been shown to alter the gut microflora [22] by reducing the number of pathogenic bacteria that colonize the gastrointestinal tract [23]. Although MOS effects are broadly studied, the specific mode of action underlying the beneficial effects of MOS remains unclear. It is suggested to involve several bacterium-related mechanisms, including pathogen exclusion through competitive binding to the mannose specific type 1 fimbriae of certain pathogens such as Escherichia coli and Campylobacter, thereby altering the gut microflora [22]. Other mechanisms have been suggested for the effects of indigestible oligosaccharides, including direct interaction of the oligosaccharides with carbohydrate receptors on intestinal epithelial cells and immune cells, and partial absorption of the oligosaccharides [24]. Hooge et al. [25] studied the effect of adding mannanoligosaccharide (MOS) with or without bacitracin (BMD) or virginiamycin (VM). They reported that the improvement in performance due to MOS was equivalent to that of BMD, and that there was an additive effect when combined with the antibiotic. By contrast, Finucane et al. [23] reported a decrease in Clostridium perfringens viable counts in young turkeys in response to including MOS or BMD in the diet but, when the additives were used in combination, the Clostridia spp. counts did not differ significantly from the control. Differences in responses may be due to intrinsic properties of the growth promoting products being added or a consequence of the experimental conditions. From a previous study [26], it appeared that the use of a combination of yeast extract and feed acidifier in a commercial diet had a positive effect on gain: feed and ME: GE. Similarly, the effects of different sources of oligosaccharide and organic acids is worthy of examination. The composition of the gut microflora plays an important role in digestion, with a beneficial, negative or neutral effect [27]. Modifications to the gastrointestinal microflora which reduce pathogen attachment may have a profound effect on the structure of the intestinal wall. However, evaluation of these feed additives efficiency which contains acidifiers and MOS on immune response and performance of broiler chicks requires studies that are more comprehensive. The aim of the present study was to investigate the effects of different dosage of a Natural Growth Promoter (Acidifier Agent) as an alternative to AGP on immune response and blood biochemical parameters of broiler chickens. The efficacy of different dosage of the acidifier was also investigated in this trial.

2. MATERIALS AND METHODS

The present study was carried out in the Department of Animal Science, Faculty of Agricultural Sciences, Malayer University, Malayer, Iran with an objective of assessing the

growth performance, immune response, and blood biochemical parameters of commercial broilers fed with acidifier.

2.1 Experimental Design, Housing, Management and Test Diet

A total number of 240 days old unsexed Ross 308 broiler chicks were wing banded, weighed and distributed in a completely randomized experimental design with four treatments and three replications of twenty chicks each. Each replicate group of chicks housed in an independent pen, conventional sided deep litter house. Chicks in all the replicates were reared up to six weeks of age under uniform standard conditions throughout the study. Brooding was done till three weeks of age using incandescent bulbs. Each pen was fitted with an automatic bell type drinker and a hanging tubular feeder. Chicks were provided with feed and water *ad libitum* throughout the study. Feeding of test diets commenced at first day of age and continued till the termination of experiment at six weeks of age. The temperature was maintained at 30±1℃ in the first week and redu ced by 2.5℃ per week to 21℃. From day one until day 4 the lighting schedule was 24 h light. At days 5-42 the dark time was increased to 1 h. Basal diet was formulated and compounded to meet the nutrient requirements of commercial broilers during the starter (0-2 wks), grower (2-4 wks) and finisher (4-6 wks) feed. The composition of experimental diets is shown in Table 1. Diets prepared without additive as Control (CON) (group1); 0.025% Acidifier Agent (AA1) (group2); 0.05% Acidifier Agent (AA2) (group 3) and 0.1% Acidifier Agent (AA3) (group 4). The natural acidifier agent used in this study was Totacid (containing citric acid, acetic acid, propionic acid, lactic acid and MOS from natural sources) provided by a commercial company (Tehran Dane Limited, Tehran, Iran).

Table 1. Ingredients and composition of the basal diets (NRC, 1994) (as-fed basis, %)

Ingredients (%)	Starting diet (0-2wk)	Growing diet (2-4wk)	Finishing diet (4-6wk)
Corm	59.00	67.36	72.01
Soybean meal	33.74	28.63	24.46
Soybean oil	1.56	0.65	0.56
Calcium carbonate	0.60	0.67	0.63
Dicalcium phosphate	1.41	1.02	0.84
Oyster shell	0.66	0.66	0.63
Common salt	0.30	0.30	0.30
Vit. And Min. Permix[1]	0.50	0.50	0.50
DL-Methionine	0.13	0.06	0.02
Lysine – HCL	0.09	0.14	0.05
Calculated analysis			
ME (Kcal/kg)	2900	2950	3000
Crude protein (%)	20.84	18.43	16.87

[1]*The vitamin and mineral premix provide the following quantities perkilogram of diet: vitamin A,10,000IU(all-trans-retinal); Vit. D3 (cholecalciferol),2,000IU; vitamin E,20IU (α-tocopherol); vitamin K3, 3.0mg; riboflavin, 18.0mg; niacin, 50mg; D-calcium pantothenic acid, 24mg; cholinechloride, 450mg; vitamin B12,0.02mg; folicacid,3.0mg;manganese,110mg;zinc,100mg;iron,60mg; copper 10mg; iodine,100mg; selenium,0.2mg and antioxidant, 250mg.*

2.2 Vaccination Schedule

Vaccination schedule was as follow:

Vaccination against Newcastle Disease (ND) virus happened three times: first spray at the commencement of experiment, second on the 12th day as B1 (CEVA SANTE ANIMALE, Libourne, France) in drinking water and booster of them on 20th day as clone-30 (HIPRAVIAR® CLON, Amer, Spain) in drinking water. Vaccination against Infectious bronchitis happened twice as the following: first spray at commencement of the experiment and the booster in drinking water on the 10th day, both as H-120 (CEVA SANTE ANIMALE, Libourne, France). Vaccination against Infection Bursal Disease (IBD) happened twice: first on day 15 and the second on the 24th day, both as Gambo-l (CEVA SANTE ANIMALE, Libourne, France) in drinking water. The sera were applied to HI test in 28 the day, to determine antibodies to NDV. In titers lower that 5, the booster B1 (CEVA SANTE ANIMALE, Libourne, France) was administrated in drinking water to broilers.

2.3 Studied Parameters

2.3.1 Immunity parameters

At the end of the trials, upon obtaining the permission of Ethical Committee of the University, six birds from each replicate were bled by jugular venipuncture and blood samples were individually collected in 10-mL heparinized tubes and stored on ice for hematological analysis. Serum was separated after 8 to 10 hours as per the standard procedures (Calnek et al. [28]) and was stored at –20ºC for subsequent analysis. The individual serum samples were analyzed for antibody titers against ND, IBD and Avian Influenza (AI) by ELISA technique. Treatment-wise means of titers were computed.

2.3.2 Biochemical parameters

The collected blood samples were analyzed for total proteins, serum albumin, uric acid and the activities of gamma glutamyltransferase (GGT) and alanine amino transferase (ALT) using automatic analyzer (Boehringer Mannheim Hitachi 704 automatic analyzer, Japan). The methodology and the set of reagents used in respect of each parameter were as recommended by the manufacturer of the analyzer system. Data are presented as means of each treatment.

2.3.3 Statistical analysis

The experimental data were analyzed statistically by using the General Linear Model procedure of the Statistical Analysis System (SAS®) software [29]. Overall data were analyzed using one way ANOVA test. Duncan multiple range test at 0.05 probability level was employed for comparison of the means [30].

3. RESULTS AND DISCUSSION

The effects of acidifier on the immune response: The results of antibody titers against ND, IBD and AI are showed in Table 2. It revealed that there was no significant difference among the dietary treatments for all titers.

Table 2. Antibody titers of broilers fed different levels of Acidifier Agent at 42 days

Treatment groups	ND	IBD	AI
[1]CON	5.00±0.29[a]	339.20±0.67[a]	1.60±0.18[a]
[2]AA1	4.90±0.84[a]	334.10±0.93[a]	1.56±0.59[a]
[3]AA2	4.88±0.23[a]	332.70±0.47[a]	1.58±0.92[a]
[4]AA3	5.04±0.58[a]	334.43±0.97[a]	1.58±0.63[a]
SEM	0.212	0.034	0.651

[1]CON (Control); [2]AA1 (Acidifier Agent @ 0.025%); [3]AA2 (Acidifier Agent @ 0.05%) and [4]AA3 (Acidifier Agent @ 0.1%, respectively). SEM: Standard Error of Mean. ND: Newcastle Disease; IBD: Infectious Bursal Disease, AI: Avian Influenza.

The effects of acidifier agent on the biochemical parameters: The results of total protein, serum albumin and serum globulin are showed in Table 3. There was no significant difference among the dietary treatments for all parameters.

Table 3. Biochemical parameters of broilers fed different levels of Acidifier Agent at 42 days

Treatment groups	Total protein (g%)	Serum albumin (g%)	Serum globulin (g%)
[1]CON	2.36±0.64[a]	1.35±0.64[a]	1.01±0.67[a]
[2]AA1	2.48±0.18[a]	1.39±0.83[a]	1.09±0.93[a]
[3]AA2	2.38±0.06[a]	1.37±0.04[a]	0.09±0.17[a]
[4]AA3	2.35±0.57[a]	1.40±0.27[a]	1.01±0.27[a]
SEM	0.281	0.145	0.241

[1]CON (Control); [2]AA1 (Acidifier Agent @ 0.025%); [3]AA2 (Acidifier Agent @ 0.05%) and [4]AA3 (Acidifier Agent @ 0.1%, respectively). SEM: Standard Error of Mean.

The effects of acidifier on the enzyme activities: The results of GGT, ALT and ALP are showed in Table 4. It was absorbed that there was no significant difference among the dietary treatments for all titers, when compared with their respective control groups. The gamma glutamyltransferase (GGT) levels varied from 9.85 to 9.97 IU/L. The values in AA3 treatment group showed slight increase in GGT value numerically. In case of alanine amino transferase (ALT), the values varied from 28.02 to 28.85 IU/L and like GGT, when compared with control group, the value of ALT in AA3 group was numerically high. Alkaline phosphatase (ALP) values ranged from 249.23 to 257.32 IU/L and no significant changes among all treatments were noticed. Likewise GGT and ALT, the ALP value for 0.1% level of acidifier was numerically higher than other dietary treatments.

Table 4. Enzyme activities of broilers fed different levels of Acidifier Agent at 42 days

Treatment groups	GGT(IU/L)	ALT(IU/L)	ALP(IU/L)
[1]CON	9.98[a]	28.02[a]	249.23[a]
[2]AA1	9.85[a]	28.24[a]	256.25[a]
[3]AA2	9.96[a]	28.81[a]	256.48[a]
[4]AA3	9.97[a]	28.85[a]	257.32[a]
SEM	0.241	0.172	0.476

Mean values within a row with different superscript letters (a, b and c) were significantly different ($p<0.05$). [1]CON (Control); [2]AA1 (Acidifier Agent @ 0.025%); [3]AA2 (Acidifier Agent @ 0.05%) and [4]AA3 (Acidifier Agent @ 0.1%, respectively). SEM: Standard Error of Mean. GGT: gamma glutamyltransferase; ALT: alanine amino transferase; ALP: Alkaline phosphatase.

No significant effect of feed additive on the immune response of broilers may be associated with the environmental condition, because this experiment was performed in an almost entirely aseptic condition. It is reported that [24,31] the mode of action of feed additives is mainly related to competitive exclusion and prevention of growth and reproduction of pathogens [32]. Accordingly, because of growth and reproduction of pathogens, high density of the birds and emergence of environmental stress, it is believed that positive effects of these feed additives may be revealed when the broilers are reared in these conditions. However, other researchers reported that organic acids improve the immune response. These researchers indicated that organic acid could stimulate immune response and increase resistance to microbial pathogens as they are utilized in broilers diet [33]. Acidifiers inhibit pathogenic bacteria adhesion to intestinal mucosa and create acidic environment in intestine [34]. Other important mechanisms which can be used in order to improve the immune level and the intestinal microfora are changing acidity of intestine through increasing concentration of lactic acid in intestine [34] and reducing activity of deleterious intestinal bacteria (*E. coli*, *Salmonella* and *Clostridium*) and increasing activity of lactobacillus. According to Savage et al. [35], it was found that the rate of lgA that comes into intestine from bile duct and also the rate of plasma lgA increases numerically, when fed with acidifier [35].

4. CONCLUSION

In conclusion, rearing broiler chickens under conditions of good hygiene, with dietary supplementation of acidifier agent did not have a clear positive effect on immune response and serum biochemical levels; although there was a slight positive effect at 0.1% level of inclusion in the diet.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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